MediGene Aktiengesellschaft

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Myocardium- and skeletal muscle-specific nucleic acid, its preparation and use

The invention relates to a nucleic acid which is expressed in human myocardium and skeletal muscle, to its preparation and use as diagnostic aid, medicinal product and test for identifying functional interactors.

The heart is a muscular hollow organ which has the task of keeping the bloodstream in the vessels in motion by alternating contraction (systole) and relaxation (diastole) of atria and ventricles.

The muscle of the heart, the myocardium, is composed of specialized striped muscle cells between which there is connective tissue. Each cell has a central nucleus, is bounded by the plasma membrane, the sarcolemma, and contains numerous contractile myofibrils which are separated irregularly by sarcoplasm. The contractile substance of the heart is formed by long parallel myofibrils. Each myofibril is divided into several identical structural and functional units, the sarcomeres. The sarcomeres in turn are composed of the thin filaments which mainly consist of actin, tropomyosin and troponin, and the thick filaments which mainly consist of myosin.

The molecular mechanism of muscle contraction is based on a cyclic attachment and detachment of the globular myosin heads by the F actin filaments. On electrical stimulation of the myocardium, Ca^{2+} is released from the sarcoplasmic reticulum which influences, through an allosteric reaction, the troponin complex and tropomyosin, and thus opens the way for contact of the actin filament with the myosin

head. The attachment causes a conformational change in the myosin which thus pulls the actin filament along on itself. ATP is needed to reverse this conformational change and return to the start of a contraction cycle.

Short-term adjustment of the activity of the myocardium to the particular perfusion requirement, that is to say blood flow requirement, of the body is possible by nervous and hormonal regulation measures. It is thus possible to increase both the force of contraction and the rate of contraction. Long-term overstrain results in physiological transformation processes in the myocardium, which are characterized mainly by an increase in myofibrils (myocyte hypertrophy).

If the myocardium is damaged, the originally physiological adaptation mechanisms often lead in the long term to pathophysiological states which develop into chronic cardiac insufficiency, that is to say cardiac weakness, and usually end with acute heart failure. In cases of severe chronic insufficiency, the heart may no longer respond appropriately to changed output requirements, and even slight physical exertion leads to exhaustion and shortness of breath.

Damage to the myocardium results from ischaemia, that is to say depletion of blood, caused by coronary disease, bacterial or viral infections, toxins, metabolic abnormalities, autoimmune diseases or genetic defects. Therapeutic measures are currently aimed at strengthening the force of contraction and controlling the compensatory neuronal and hormonal compensation mechanisms. Despite this treatment, the mortality from this disease remains high (35-50% in the first 5 years after diagnosis). Cardiac insufficiency is the main cause of death in the world. The only causal therapy is a heart transplant.

The molecular changes in chronic cardiac insufficiency are only inadequately known. In

particular, the genetic changes underlying cardiac insufficiency are substantially unknown. The question of why secondary damage by toxins or viruses leads to cardiac insufficiency in some people but not in others also remains unanswered.

Summary of the Invention

The present invention is ~~thus~~ based on the object of identifying and isolating genes which are at least partly responsible for, if not in fact the causes of, genetically related cardiac disorders.

Surprisingly, a gene has now been found, in a human cardiac tissue cDNA bank, which is expressed more strongly in insufficient cardiac tissue than in healthy cardiac tissue and thus is causally connected with a genetically related cardiac insufficiency. A so-called EST (expressed sequence tag) already exists for this gene, although it is faulty and no function at all can be assigned to it (Tanaka, T. et al. (1996) Genomics, 35, 231-235; EMBL AC:CO4498; clone 3NHC3467).

One aspect of the invention is therefore a nucleic acid coding for a polypeptide having an amino acid sequence as shown in Fig. 4 (SEQ ID NO:4) or a functional variant thereof, and parts thereof having at least 8 nucleotides, preferably at least 10 nucleotides, in particular at least 15 nucleotides, especially at least 20 nucleotides, except a nucleic acid having the sequence: (SEQ ID NO:6)

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1   GCCAACACGC ANTCCGACGA CAGTGCAGCC ATGGTCATTG CAGAGATGCN TCAAAGTCAA
61  TGAGCACATC ACCAACGTAA ACGTCGAGTC CAACTTCATA ACGGGAAAGG GGATCCTGGC
121 CATCATGAGA GCTCTCCAGC ACAACACGGT GCTCACGGAG CTGCGTTTCC ATAACCAGAG
181 GCACATCATG GGCAGCCAGG TGGAAATGGA GATTGTCAAG CTNCTGAAGG AGAACACGAC
241 GCTNCTGAGG CTGGGNTACC ATTTTNAACT CCCAGGACC
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in which N denotes A, T, G or C.

The nucleic acid according to the invention was isolated from a human cardiac tissue cDNA bank and sequenced. For this, firstly complete RNA was isolated by standard methods from a healthy and insufficient cardiac sample and transcribed with the aid of a

3'anchor primer mixture, for example a 5'-T_{12}ACN-3' primer, in which N denotes any deoxyribonucleotide, and reverse transcriptase into c-DNA. The cDNA was then amplified with a method based on the so-called differential display method of Liang and Pardee (Liang, P. & Pardee, A. (1992) *Science* 257, 967-970) under specific PCR conditions with the aid of a 3' primer, for example a T_{12}ACN primer, and of an arbitrarily selected 5'-decamer primer, for example a 5'-CCTTCTACCC-3' decamer primer (SEQ ID NO: 5). It was possible thereby to amplify a 321 base pair (bp)-long DNA fragment which is surprisingly present not in the healthy heart sample but distinctly in the insufficient heart sample. This was surprising because the conventional methods such as the differential display method or else subtractive cDNA gene banks are associated with the problem of redundancy, of under-representation and of false-positive clones. In particular, it is possible to identify the gene products of weakly expressed genes only under special conditions. It is therefore also not astonishing that the hit rate is generally very low (10-20%) and, for example in the differential display method, also depends on the chosen PCR conditions, the primer length or, for example in the production of subtractive banks, on the hybridization temperature. The complete gene was then isolated from a cDNA gene bank with the aid of the found DNA fragment and sequenced.

In every case it is necessary to find out by further methods whether the found cDNA can be assigned to an active and/or tissue-specific gene. Hence mRNAs from various human tissues were hybridized with the found DNA fragment in a so-called Northern blot, and the amount of bound m-RNA was determined, for example, via the radiolabelling of the DNA fragment. This experiment led to detection of the corresponding RNA in particular in striped muscle, that is to say myocardial

and skeletal muscle tissue, and very weakly in prostate tissue. In a further experiment comparing between healthy and insufficient cardiac tissues, increased expression was detected, for example expression of the RNAs increased by about 35%, in insufficient tissue by comparison with healthy tissue. It was possible to demonstrate in particular that a relatively small RNA species preferentially shows increased expression in insufficient tissue by comparison with healthy tissue. The increased expression of the relatively small RNA species is readily evident for example in the Northern blot in the form of a double band (see Fig. 5b).

Comparison of the derived polypeptide sequence with a protein database additionally revealed a certain relationship (homology) with the protein tropomodulin (see Fig. 4). Tropomodulin is known to be a polypeptide which in chicken cardiomyocytes has an influence on the development of the myofibrils and the contractility of the cells (Gregorio et al. (1995) *Nature* 377, 83-86). This protein binds on the one hand to tropomyosin, and on the other hand to the actin filaments, but is not itself regulated in its activity. The derived polypeptide according to the invention likewise has some of the structural features of tropomodulins, such as, for example, a tropomyosin binding domain. In contrast to tropomodulin, the polypeptide according to the invention has additional structural features indicating regulation of the activity of the polypeptide by so-called tyrosine kinases (see Fig. 4).

The term "functional variant" therefore means for the purpose of the present invention polypeptides which are functionally related to the polypeptide according to the invention, that is to say can likewise be referred to as a regulable modulator of the contractility of myocardial cells, are expressed in striped muscle, preferably in myocardial, skeletal muscle and/or prostate tissue, especially in myocardial

and/or skeletal muscle and, in particular, in myocardial cells, have structural features of tropomodulin, such as, for example, one or more tropomyosin binding domains, and/or whose activity can be regulated by tyrosine kinases. Examples of functional variants are the corresponding polypeptides derived from other organisms than humans, preferably from non-human mammals such as, for example, monkeys.

In the wider sense, the term „~~functional variant~~" includes polypeptides which have a sequence homology, in particular a sequence identity, of about 70%, preferably about 80%, in particular about 90%, especially about 95%, with the polypeptide having the amino acid sequence shown in Fig. 4. These include, for example, polypeptides encoded by a nucleic acid which is isolated from non-heart-specific tissue, for example skeletal muscle tissue, but which has, after expression in a heart-specific cell, the identified function(s). These furthermore include deletions of the polypeptide in the region of about 1-60, preferably of about 1-30, in particular of about 1-15, especially of about 1-5, amino acids. For example, the first amino acid methionine can be absent with negligible alteration in the function of the polypeptide. These also include fusion proteins which comprise the above-described polypeptides according to the invention, it being possible for the fusion proteins themselves to have the function of a regulable modulator of the contractility of myocardial cells, or to acquire the specific function only after elimination of the fusion portion. They particularly include fusion proteins with a content of, in particular, non-heart-specific sequences of about 1-200, preferably about 1-150, in particular about 1-100, especially about 1-50, amino acids. Examples of non-heart-specific peptide sequences are prokaryotic peptide sequences which may be derived, for example, from the galactosidase of E. coli.

The nucleic acid according to the invention is generally a DNA or RNA, preferably a DNA. Preferred for expression of the relevant gene is in general a double-stranded DNA and for use as probe is a single-stranded DNA. Particular preference is given to a double- or single-stranded DNA having a nucleic acid sequence as shown in Fig. 1, 2 or 3 and the parts thereof described in detail above, with the DNA region coding for the polypeptide being particularly preferred. This region starts with the nucleic acids "ATG" coding for methionine at position 89 to "TAG" coding for "amber" (stop) at position 1747.

The nucleic acid according to the invention can, for example, be chemically synthesized on the basis of the sequences disclosed in Figs. 1-3 or (SEQ ID NOS: 1-3) on the basis of the polypeptide sequence disclosed in Fig. 4 by use of the genetic code, for example by the phosphotriester method (see, for example, Uhlmann, E. & Peyman, A. (1990) *Chemical Reviews*, 90, 543-584, No. 4). Another possibility for obtaining the nucleic acid according to the invention is isolation from a suitable gene bank, for example from a heart-specific gene bank, using a suitable probe (see, for example, J. Sambrook et al., (1989), *Molecular Cloning. A Laboratory Manual* 2nd edn., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY). Suitable as ~~probe~~ probes are, for example, single-stranded DNA fragments with a length of about 100-1000 nucleotides, preferably with a length of about 200-500 nucleotides, in particular with a length of about 300-400 nucleotides, whose sequence can be derived from the nucleic acid sequences shown in Figs. 1-3. (SEQ ID NOS: 1-3) One example of a probe is the DNA fragment of Example 1, which is 321 bp in size and corresponds to the underlined region in Fig. 1, (SEQ ID NO: 1) using which the nucleic acid according to the invention has already been isolated successfully from human cardiac tissue (see Example 2).

The nucleic acid according to the invention is normally present in a vector, preferably in an expression vector or vector effective for gene therapy. The vector effective for gene therapy preferably contains heart-specific regulatory sequences such as, for example, the troponin C (cTNC) promoter (see, for example, Parmacek, M.S. et al. (1990) *J. Biol. Chem.* 265 (26) 15970-15976 and Parmacek, M.S. et al. (1992) *Mol. Cell Biol.* 12(5), 1967-1976), which is functionally connected to the nucleic acid according to the invention.

The expression vectors may be prokaryotic or eukaryotic expression vectors. Examples of prokaryotic expression vectors for expression in E. coli are, for example, the vectors pGEM or pUC derivatives, and of eukaryotic expression vectors for expression in Saccharomyces cerevisiae are, for example, the vectors p426Met25 or p426GAL1 (Mumberg et al. (1994) Nucl. Acids Res., 22, 5767-5768) for expression in insect cells are, for example, baculovirus vectors as disclosed in EP-B1 0 127 839 or EP-B1 0 549 721, and for expression in mammalian cells are, for example, the vectors Rc/CMV and Rc/RSV or SV40 vectors, which are all generally available.

The expression vectors generally also contain promoters suitable for the particular host cell, such as, for example, the trp promoter for expression in E. coli (see, for example, EP-B1 0 154 133), the ADH2 promoter for expression in yeasts (Russell et al. (1983), *J. Biol. Chem.* 258, 2674-2682), the baculovirus polyhedrin promoter for expression in insect cells (see, for example, EP-B1 0 127 839) or the SV40 early promoter or LTR promoters, for example of MMTV (mouse mammary tumour virus; Lee et al. (1981) *Nature* 214, 228-232).

Examples of vectors effective for gene therapy are virus vectors, preferably adenovirus vectors, in

particular replication-deficient adenovirus vectors, or adeno-associated virus vectors, for example an adeno-associated virus vector which consists exclusively of two inverted terminal repeats (ITR).

An adenovirus vector and, in particular, a replication-deficient adenovirus vector are particularly preferred for the following reasons.

The human adenovirus belongs to the class of double-stranded DNA viruses with a genome of about 36 kilobase pairs (kb). The viral DNA codes for about 2700 different gene products, a distinction being made between early (*"early genes"*) and late (*"late genes"*). The *"early genes"* are divided into four transcriptional units E1 to E4. The late gene products code for the capsid proteins. It is possible to distinguish immunologically at least 42 different adenoviruses and subgroups A to F, all of which are suitable for the present invention. A precondition for transcription of the viral genes is expression of the E1 region which codes for a transactivator of adenoviral gene expression. This dependence of the expression of all subsequent viral genes on the E1 transactivator can be utilized to construct adenoviral vectors not capable of replication (see, for example, McGrory, W.J. et al. (1988) *Virol.* 163, 614-617 and Gluzman, Y. et al. (1982) in *"Eukaryotic Viral Vectors"* (Gluzman, Y. ed.) 187 - 192, Cold Spring Harbor Press, Cold Spring Harbor, New York). In adenoviral vectors, especially of type 5 (for sequence, see Chroboczek, J. et al. (1992) *Virol.* 186, 280-285) and especially of subgroup C, in general the E1 gene region is replaced by a foreign gene with its own promoter or by the nucleic acid construct according to the invention. Replacement of the E1 gene region which is a precondition for expression of the downstream adenoviral genes results in an adenovirus not capable of replication. These

viruses are then able to replicate only in a cell line which replaces the missing E1 genes.

Replication-deficient adenoviruses are therefore generally formed by homologous recombination in the so-called 293 cell line (human embryonic kidney cell line) which has a copy of the E1 region stably integrated into the genome. For this purpose, the nucleic acid according to the invention is cloned into recombinant adenoviral plasmids under the control of its own promoter, for example the troponin C promoter mentioned above. Homologous recombination then takes place with an E1-deficient adenoviral genome such as, for example, d1327 or del1324 (adenovirus 5) in the 293 helper cell line. Where recombination is successful, viral plaques are harvested. The replication-deficient viruses produced in this way are employed in high titres (for example 10^9 to 10^{11} plaque forming units) for infecting the cell culture or for somatic gene therapy.

The exact site of insertion of the nucleic acid according to the invention into the adenoviral genome is in general not critical. It is, for example, also possible to clone the nucleic acid according to the invention in place of the deleted E3 gene (Karlsson, S. et al. (1986), *EMBO J.* 5, 2377 - 2385).

However, it is preferred for the *E*1 region or parts thereof, for example the *E*1A or *E*1*B* region (see, for example, *WO* 95/00655), to be replaced by the nucleic acid according to the invention, especially when the *E*3 region is also deleted.

However, the present invention is not confined to the adenoviral vector system; on the contrary, adeno-associated virus vectors are also particularly suitable in combination with the nucleic acid according to the invention for the following reasons.

The AAV virus belongs to the family of parvoviruses. These are distinguished by an

icosahedral, non-enveloped capsid which has a diameter of 18 to 30 nm and which contains a linear, single-stranded DNA of about 5 kb. For efficient replication of AAV, coinfection of the host cell with helper viruses is necessary. Examples of suitable helpers are adenoviruses (Ad5 or Ad2), herpesviruses and vaccinia-viruses (Muzyczka, N. (1992) *Curr. Top. Microbiol. Immunol.* 158, 97-129). In the absence of a helper virus, AAV passes into a latency state where the virus genome is able to integrate stably into the host cell genome. The property of AAV integrating into the host genome makes it particularly interesting as transduction vector for mammalian cells. Generally sufficient for the vector functions are the two inverted terminal repeats (ITR: see, for example, WO 95/23867) which are about 145 bp long. They carry the signals necessary in "cis" for replication, packaging and integration into the host cell genome. For packaging into recombinant vector particles, a vector plasmid which harbours the genes for non-structural proteins (rep proteins) and for structural proteins (cap proteins) is transfected into adenovirus-infected cells. After a few days, a cell-free lysate containing, besides the recombinant AAV particles, also adenoviruses is prepared. The adenoviruses can advantageously be removed by heating at 56°C or by banding in a caesium chloride gradient. It is possible with this cotransfection method to achieve rAAV titres of 10^5 to 10^6 IE/ml. Contamination by wild-type viruses is below the detection limit if the packaging plasmid and the vector plasmid have no overlapping sequences (Samulski, R.J. (1989) *J. Virol.* 63, 3822 - 3828).

Transfer of the nucleic acid according to the invention into somatic cells can be effected by AAV into resting, differentiated cells, which is particularly advantageous for gene therapy of the heart. The ability to integrate which has been

mentioned also ensures long-lasting gene expression in vivo, which in turn is particularly advantageous. A further advantage of AAV is that the virus is not pathogenic for humans and is relatively stable in vivo. Cloning of the nucleic acid according to the invention into the AAV vector or parts thereof takes place by methods known to the skilled person, as described, for example, in WO 95/23867, in Chiorini, J.A. et al. (1995), *Human Gene Therapy* 6, 1531-1541 or Kotin, R.M. (1994), *Human Gene Therapy* 5, 793-801.

Vectors effective for gene therapy can also be obtained by complexing the nucleic acid according to the invention with liposomes, because it is possible thereby to achieve a very high transfection efficiency, in particular of myocardial cells (Felgner, P.L. et al. (1987), *Proc. Natl. Acad. Sci USA* 84, 7413-7417). In lipofection, small unilamellar vesicles of cationic lipids are prepared by ultrasound treatment of the liposome suspension. The DNA is bound ionically to the surface of the liposomes, specifically in a ratio such that a positive net charge remains and the plasmid DNA is 100% complexed by the liposomes. Besides the lipid mixtures DOTMA (1,2-dioleoyloxypropyl-3-trimethyl-ammonium bromide) and DOPE (dioleoylphosphatidyl-ethanolamine) employed by Felgner et al. (1987, supra), numerous new lipid formulations have now been synthesized and tested for their efficiency in transfecting various cell lines (Behr, J.P. et al. (1989), *Proc. Natl. Acad. Sci. USA* 86, 6982-6986; Felgner, J.H. et al. (1994) *J. Biol. Chem.* 269, 2550-2561; Gao, X. & Huang, L. (1991), *Biochim. Biophys. Acta* 1189, 195-203). Examples of the novel lipid formulations are DOTAP N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium ethyl sulphate or DOGS (TRANSFECTAM; dioctadecyl-amidoglycylspermine). One example of the preparation of DNA-liposome complexes and successful use thereof in

heart-specific transfection is described in DE 44 11 402.

For use of the nucleic acid according to the invention in gene therapy, it is also advantageous if the part of the nucleic acid which codes for the polypeptide contains one or more noncoding sequences, including intron sequences, preferably between the promoter and the start codon of the polypeptide, and/or a polyA sequence, in particular the naturally occurring polyA sequence or an SV40 virus polyA sequence, especially at the 3' end of the gene, because this makes it possible to stabilize the mRNA in the myocardial cell (Jackson, R.J. (1993) *Cell* 74, 9-14 and Palmiter, R.D. et al. (1991) *Proc. Natl. Acad. Sci. USA* 88, 478-482).

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The present invention further relates to the polypeptide itself having an amino acid sequence as shown in Fig. 4 or a functional variant thereof, and parts thereof having at least 6 amino acids, preferably having at least 12 amino acids, in particular having at least 15 amino acids and especially having at least 164 amino acids, except a polypeptide having the sequence: (SEQ ID NO:

PTRNPTTVQPWSLQRCIKVNEHITNVNVESNFITGKGILAIMRALQ

10 20 30 40

HNTVLTELRFHNQRHIMGSQVEMEIVKLLKENTTLLRLGYHFKLPG

50 60 70 80 90

The polypeptide is prepared, for example, by expression of the nucleic acid according to the invention in a suitable expression system as described above using methods generally known to the skilled person. Examples of suitable host cells are the E. coli strains DH5, HB101 or BL21, the yeast strain Saccharomyces cerevisiae, the lepidopteran insect cell line for example Spodoptera frugiperda, or the animal

cells COS, Vero, 293 and HeLa, all of which are generally obtainable.

The said parts of the polypeptide can also be synthesized by classical synthesis (Merrifield technique). They are particularly suitable for obtaining antisera which can be used to screen suitable gene expression banks in order thus to obtain further functional variants of the polypeptide according to the invention.

The present invention therefore relates also to antibodies which react specifically with the polypeptide having an amino acid sequence as shown in Fig. 4 (SEQ ID NO:4) or a functional variant thereof, and parts thereof having at least 6 amino acids, preferably having at least 12 amino acids, in particular having at least 15 amino acids and especially having at least 164 amino acids, the abovementioned parts of the polypeptide either themselves being immunogenic or being able to be made immunogenic, or have their immunogenicity increased, by coupling to suitable carriers such as, for example, bovine serum albumin.

The antibodies are either polyclonal or monoclonal. The preparation, to which the present invention also relates, takes place, for example, by generally known methods, by immunizing a mammal, for example a rabbit, with the said polypeptide or the said parts thereof, where appropriate in the presence of, for example, Freund's adjuvant and/or aluminium hydroxide gels (see, for example, Diamond, B.A. et al. (1981) *The New England Journal of Medicine*, 1344-1349). The polyclonal antibodies raised in the animal on the basis of an immunological response can then easily be isolated from the blood by generally known methods and purified, for example, by column chromatography. It was thus possible, for example, to produce in rabbits a polyclonal antiserum against a polypeptide which had amino acids 1-90 according to the invention, as shown

in Fig. 4 (SEQ ID NO:4), which was expressed as fusion protein in bacteria and purified by affinity chromatography. The antibodies according to the invention specifically recognized the corresponding protein of about 80 kD in extracts of human heart tissue.

Monoclonal antibodies can be prepared, for example, by the known method of Winter & Milstein (Winter, G. & Milstein, C. (1991) *Nature*, 349, 293-299).

The present invention also relates to a medicinal product which contains a nucleic acid coding for a polypeptide having an amino acid sequence as shown in Fig. 4 or a functional variant thereof and the abovementioned parts thereof having at least 8 nucleotides, or a polypeptide having an amino acid sequence as shown in Fig. 4 or a functional variant thereof and the abovementioned parts thereof having at least 6 amino acids and, where appropriate, suitable additives or excipients, and to a process for producing a medicinal product for treating cardiac disorders, in particular cardiac insufficiency, in which a said nucleic acid or a said polypeptide is formulated with a pharmaceutically acceptable carrier.

One example of the use of nucleic acid fragments as therapeutic ~~agent~~ Agents is the use of DNA fragments in the form of antisense oligonucleotides (Uhlmann, E. & Peyman, A. (1990) Chemical Reviews, 90, 543-584, No. 4).

A particularly suitable medicinal product for use for human gene therapy is one which contains the said nucleic acid in naked form or in the form of one of the vectors effective for gene therapy which are described above, or in a form complexed with liposomes. The pharmaceutical carrier is, for example, a physiological buffer solution, preferably with a pH of about 6.0-8.0, preferably of about 6.8-7.8, in particular of about 7.4 and/or an osmolarity of about

200-400 milliosmol/litre, preferably of about 290-310 milliosmol/litre. The pharmaceutical carrier may additionally contain suitable stabilizers such as, for example, nuclease inhibitors, preferably complexing agents such as EDTA and/or other excipients known to the skilled person.

The said nucleic acid is normally administered intravenously, for example with the aid of a catheter, where appropriate in the form of the virus vectors described in detail above or as liposome complexes. It is advantageous, for example, to infuse the nucleic acid according to the invention directly into the patient's coronary arteries (so-called percutaneous coronary gene transfer, PCGT), in particular in the form of recombinant adenovirus vectors or adeno-associated virus vectors. Administration with the aid of a balloon catheter is particularly preferred because it is possible thereby to confine the transfection not only to the heart but also to the injection site within the heart (see, for example, Feldman, L.J. et al. (1994) *JACC* 235A, 906-934).

It is also possible to administer the polypeptide itself intravenously or with the aid of a catheter or balloon catheter, where appropriate with suitable additives or excipients, such as, for example, physiological saline, stabilizers, proteinase inhibitors etc., in order to influence the function of the heart immediately and directly.

The present invention further relates to a diagnostic aid containing a nucleic acid, a polypeptide or antibody according to the present invention and, where appropriate, suitable additives or excipients and to a process for producing a diagnostic aid for diagnosing cardiac disorders, in particular cardiac insufficiency, in which a nucleic acid, a polypeptide or antibody according to the present invention is mixed with suitable additives or excipients.

It is possible, for example, according to the present invention to produce on the basis of the said nucleic acid a diagnostic aid based on the polymerase chain reaction (PCR diagnosis, for example as disclosed in EP-0 200 362) or on a Northern blot as described in detail in Example 3 using the 321 bp DNA fragment according to the invention as probe. These tests are based on the specific hybridization of said nucleic acids with the complementary strand, normally of the corresponding mRNA. The nucleic acid may also in this case be modified as described, for example, in EP 0 063 879. A DNA fragment, in particular the DNA fragment described in Example 1, is preferably labelled using suitable reagents, for example radioactively with α-^{32}P-dCTP or non-radioactively with biotin, by generally known methods and incubated with isolated RNA, which has preferably been pre-bound to suitable membranes made of, for example, cellulose or nylon. It is additionally advantageous, before the hybridization and binding to a membrane, for the isolated RNA to be fractionated according to size, for example by agarose gel electrophoresis. With the same amount of investigated RNA from each tissue sample, it is thus possible to determine the amount of mRNA specifically labelled by the probe.

It is thus possible by using the diagnostic aid according to the invention also to measure a cardiac tissue sample in vitro specifically for the strength of expression of the corresponding gene in order to be able to diagnose reliably possible cardiac insufficiency (see Example 1). A cDNA having a sequence as shown in Fig. 1 is particularly suitable for diagnosing a possible cardiac insufficiency (see Example 2).

A further diagnostic aid contains the polypeptide according to the present invention or the immunogenic parts thereof described above in detail.

The polypeptide or the parts thereof, which are preferably bound to a solid phase, for example made of nitrocellulose or nylon, can, for example, be brought into contact in vitro with the body fluid to be investigated, for example blood, in order to react for example with autoimmune antibodies. The antibody-peptide complex can then be detected for example by means of labelled antihuman IgG or antihuman IgM antibodies. The label is, for example, an enzyme such as peroxidase which catalyses a colour reaction. The presence and the amount of autoimmune antibody present can thus be detected easily and rapidly by the colour reaction.

Another diagnostic aid contains the antibodies according to the invention themselves. These antibodies can be used, for example, for investigating a cardiac tissue sample easily and quickly to find whether the relevant polypeptide is present in an increased amount, in order thus to obtain information about possible cardiac insufficiency. In this case, the antibodies according to the invention are labelled for example with an enzyme, as already described above. The specific antibody-peptide complex can thus be detected easily and equally quickly by an enzymatic colour reaction.

The present invention also relates to a test for identifying functional interactors containing a nucleic acid according to the invention coding for a polypeptide having an amino acid sequence as shown in Fig. 4 (SEQ ID NO:4) or a functional variant thereof and the abovementioned parts thereof having at least 8 nucleotides, a polypeptide having the amino acid sequence as shown in Fig. 4 (SEQ ID NO:4) or a functional variant thereof, and the abovementioned parts thereof having at least 6 amino acids or the antibodies according to the invention and, where appropriate, suitable additives or excipients.

A suitable test for identifying functional interactors is, for example, the so-called two-hybrid system (Fields, S. & Sternglanz, R. (1994) *Trends in Genetics*, 10, 286-292).

In this test, a cell, for example a yeast cell, is transformed or transfected with one or more expression vectors which express a fusion protein which contains a polypeptide according to the present invention and a DNA binding domain of a known protein, for example of Gal4 or LexA from E. coli, and/or expresses a fusion protein which contains an unknown polypeptide and a transcription activating domain, for example of Gal4, herpes virus VP16 or B42. The cell additionally contains a reporter gene, for example the lacZ gene from E. coli, green fluorescence protein or the yeast amino acid biosynthesis genes His3 or Leu2, which is controlled by regulatory sequences, such as, for example, the LexA promoter/operator or by a so-called upstream activation sequence (UAS) of yeast. The unknown polypeptide is encoded, for example, by a DNA fragment which is derived from a gene bank, for example from a human cardiac tissue-specific gene bank. Normally a cDNA gene bank is produced directly, using the expression vectors described, in yeast so that the test can be carried out immediately thereafter.

For example, a nucleic acid according to the present invention is cloned into a yeast expression vector in a functional unit with the nucleic acid coding for the LexA DNA binding domain, so that a fusion protein consisting of the polypeptide according to the invention and the LexA DNA binding domain is expressed in the transformed yeast. In another yeast expression vector, cDNA fragments from a cDNA gene bank are cloned in a functional unit with the nucleic acid coding for the Gal4 transcription activating domain, so that a fusion protein consisting of an unknown polypeptide and the Gal4 transcription activating

domain is expressed in the transformed yeast. The yeast which is transformed with the two expression vectors and is, for example, Leu2$^-$ additionally contains a nucleic acid which codes for Leu2, and is controlled by the LexA promoter/operator. In the event of a functional interaction between the polypeptide according to the invention and the unknown polypeptide, the Gal4 transcription activating domain binds via the LexA DNA binding domain to the LexA promoter/operator, whereby the latter is activated and the Leu2 gene is expressed. The result of this is that the Leu2$^-$ yeast is able to grow on minimal medium which contains no leucine.

On use of the lacZ or green fluorescence protein reporter gene in place of an amino acid biosynthesis gene, activation of transcription can be detected by the formation of blue or green-fluorescing colonies. The blue or fluorescent coloration can also be quantified easily in a spectrophotometer, for example at 585 nm in the case of a blue coloration.

Thus, it is possible to screen expression gene banks easily and quickly for polypeptides which interact with a polypeptide according to the present invention. It is then possible for the novel peptides found to be isolated and further characterized.

Another possible use of the two-hybrid system is the influence on the interaction between a polypeptide according to the present invention and a known or unknown polypeptide by other substances such as, for example, chemical compounds. Thus, it is also possible to find easily novel and valuable active substances which can be chemically synthesized and can be employed as therapeutic ~~agent~~ Agent C for treating a cardiac disorder. The present invention is therefore not restricted to a method for finding polypeptide-like interactors, but also extends to a method for finding substances which are able to interact with the protein-

protein complex described above. Such polypeptide-like, as well as chemical interactors are therefore referred to as functional interactors for the purpose of the present invention.

The surprising advantage of the present invention is thus the possibility of using the subject-matters according to the invention for specific and reliable diagnosis and therapy of cardiac disorders, especially cardiac insufficiency. However, other valuable therapeutic and diagnostic possibilities also emerge. For example, the functional interactors which can be easily identified using the described test methods are so advantageous because it is possible with their aid in the form of suitable medicinal products to influence deliberately the activity of the polypeptide according to the invention in its natural environment in the myocardium and thus also the contractility of the myocardial cells, in particular since the activity of this polypeptide can be regulated as already described in detail above.

The following figures and examples are intended to illustrate the invention in detail without restricting it.

Detailed Description

~~Description of the figures~~

Sub C3

Fig. 1 shows a 1936 nucleotide-long heart-specific DNA sequence. The region which codes for the corresponding polypeptide is shown in bold. The DNA fragment from Example 1 is underlined.

Fig. 2 shows a 2080 nucleotide-long heart-specific DNA sequence which has an extension at the 5' end of the DNA sequence from Fig. 1. The region which codes for the corresponding polypeptide is once again shown in bold.

Fig. 3 shows a 2268 nucleotide-long heart-specific DNA sequence which has an extension at the 5' end of the DNA sequence from Fig. 1 or Fig. 2. The

region which codes for the corresponding polypeptide is likewise shown in bold.

Fig. 4 shows a 552 amino acid-long polypeptide sequence encoded by one of the DNA sequences shown in Figs. 1-3. The regions homologous with human tropomodulin are shown in bold. The sequence motifs which indicate regulation of the polypeptide by tyrosine kinase signal transduction pathways are underlined.

Figs. 5a and 5b show Northern blots of mRNAs which correspond to the nucleic acid sequences shown in Figs. 1-3 for detecting expression in various human tissues (Fig. 5a) and for detecting expression in healthy and insufficient human cardiac tissue (Fig. 5b).

Examples

1. Isolation of a DNA fragment from human insufficient cardiac tissue

Complete RNA was initially isolated by standard methods (Chomczynski & Sacchi (1987), *Anal. Biochem*, 162 (1), 156-159) from a healthy and an insufficient cardiac tissue sample. The RNA was then treated with DNAse in order to remove DNA contamination. An aliquot of this RNA (0.2 µg) was then incubated in a 20 µl reaction mix with 1 × RT buffer (Gibco Y00121), 10 mM DTT, 20 µM dNTP mix, 1 U/µl RNAsin (Promega N2511), 1 µM 3' anchor primer mixture of the 5'-T_{12}ACN-3' type, where N can be any deoxynucleotide, and 10 U/µl SuperScript RNAse H$^-$ reverser transcriptase at 37°C for 60 min and thus transcribed into cDNA. A cDNA aliquot was then subjected to a 20 µl PCR in 1 × PCR buffer (Perkin-Elmer) which, besides 1 µm 3' primer T_{12}AC and 1 µM 5'-decamer primer (5'-CCTTCTACCC-3'), contains 10 µCi of α-^{32}P-dCTP, 2 µM dNTP mix and 1 U of AmpliTaq (Perkin Elmer). The mixture was incubated firstly at 94°C for 1 min, then 40 cycles each of 30 s at 94°C,

40°C for 2 min and 72°C for 30 s and finally at 72°C for 10 min. The resulting DNA fragment mixture was then fractionated on a 6% polyacrylamide gel and autoradiographed. A DNA fragment which is 321 bp in length and which is not present in the healthy heart sample but is distinctly present in the insufficient heart sample is thus prepared. This fragment was then cut out of the gel on the basis of the X-ray film and was reamplified by PCR under the conditions already described. The resulting fragment was then cloned into an appropriate vector, and the DNA sequence was determined. A fragment prepared in this way contains nucleotides 1627-1936 of the sequence according to Claim 1 and the 12 thymine nucleotides from the 3' ~~anchor primer~~.

2. Isolation of heart-specific nucleic acids

A plaque hybridization was carried with a cDNA gene bank from cardiac tissue under standard conditions (see Sambrook, J., Frisch, E.F. & Maniatis, T. (1989) *Molecular Cloning, A Laboratory Manual*, ch. 8-10) using an α-^{32}P-dCTP-labelled DNA fragment from Example 1 which comprises the nucleotides from position 1627-1936 in Fig. 1. (SEQ ID NO:1) The cDNAs found were then isolated and sequenced. The sequences are shown in Figs. 1-3. (SEQ ID NOS:1-3) It emerged from this that the cDNA having the sequence shown in Fig. 1 (SEQ ID NO:1) could be isolated with greater probability from insufficient cardiac tissue than the cDNA having the sequence shown in Fig. 2 or 3, (SEQ ID NO:2) (SEQ ID NO:3) which could be isolated with greater probability from healthy cardiac tissue.

3. Detection of the strength of expression of the heart-specific gene in various human tissues by means of Northern blots.

The DNA fragment 321 bp in length already described in Examples 1 and 2 and Fig. 1 (SEQ ID NO:1) was firstly

radiolabelled with α-^{32}P-dCTP by the random primer labelling method (Feinberg, A.P. & Vogelstein, B. (1983) Anal. Biochem., 132, 6). The RTS RadPrime DNA labelling system (GibcoBRL 10387-017) was used for this purpose. The hybridization of blots with poly A$^+$ RNA from human tissues (see Figs. 5a and 5b) took place at 68°C for 1 hour in accordance with the manufacturer's instructions (Multiple Tissue Northern Blots I & II, Clontech Laboratories GmbH, Heidelberg, #7760-1, #7759-1) in ExpressHyb hybridization solution (Clontech #8015-1). The blots were then washed with 2 x SSC and 0.05% SDS for 30 minutes and thereafter with 0.1 x SSC and 0.1% SDS for 1 hour and autoradiographed. It emerged that the probe 321 bp in length hybridizes strongly with a polyA$^+$ RNA of about 2400 bp strongly in cardiac tissue and skeletal muscle, very weakly in prostate tissue and not in leucocytes, large intestinal, small intestinal, ovarian, testicular, thymus, splenic, renal, hepatic, lung, placental and brain tissue (Fig. 5a).

Expression of the corresponding RNAs in healthy and insufficient cardiac tissue was also investigated. Complete RNA was isolated from various human cardiac tissue samples for this purpose (Chomczynski & Sacchi (1987), Anal. Biochem. 162, 156-159). Subsequently in each case 10 µg of RNA were fractionated using a 1% formaldehyde agarose gel and transferred by the capillary method to a charged nylon membrane (Zeta-Probe GT BioRad #162-0197). The membrane was briefly washed with 2 x SSC and then baked at 80°C for 30 minutes. The membranes were incubated with prehybridization solution (0.5 M Na_2HPO_4, pH 7.2; 7% SDS) at 65°C for at least 1 hour. The solution was then replaced by a fresh solution, and the radioactive, heat-denatured probe was added. The hybridization was carried out at 65°C for 15 hours. The membranes were then washed firstly with 40 mM Na_2HPO_4, pH 7.2; 5% SDS

at 65°C for 15 hours and then with 40 mM Na_2HPO_4, pH 7.2; 1% SDS at 65°C for 2 × 30 minutes, and subsequently autoradiographed. It emerged that various RNA species having a length from about 2200 to 2400 bp were fractionated in 1% agarose gels. These different species correspond well with the sizes of the three cDNAs found, including an average polyA tail 150 bp long (see Figs. 1-3) (SEQ ID NOS: 1-3). In particular, the smallest RNA species was more clearly detectable in diseased tissue than in healthy tissue. Quantification of the blot using a PhosphorImager and the ImageQuant software (Molecular Dynamics GmbH, Krefeld), taking into account a control hybridization with β4-thymosin and actin, revealed an approximately 35% increased expression of the detected RNAs in insufficient cardiac tissue by comparison with healty tissue.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: MediGene Aktiengesellschaft
(B) STREET: Lochhamer Str. 11
(C) CITY: 82152 Martinsried
(D) COUNTRY: Germany
(F) POSTAL CODE: D-82152
(G) TELEPHONE: 089-89 56 32 0
(H) FAX: 089-89 56 32 20

(ii) TITLE OF INVENTION: Myocardium- and skeletal muscle-specific nucleic acid, its preparation and use

(iii) NUMBER OF SEQUENCES: 5

(iv) COMPUTER-READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: Word Perfect 3.1

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1936 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: YES

(vi) ORIGINAL SOURCE:

(F) TISSUE TYPE: cardiac tissue

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 1:

CAGCCTGCCA CTTGCCTCCC TGCCTGCTTC TGGCTGCCTT GAATGCCTGG TCCTTCAAGC 60

TCCTTCTGGG TCTGACAAAG CAGGGACCAT GTCTACCTTT GGCTACCGAA GAGGACTCAG 120

TAAATACGAA TCCATCGACG AGGATGAACT CCTCGCCTCC CTGTCAGCCG AGGAGCTGAA 180

GGAGCTAGAG AGAGAGTTGG AAGACATTGA ACCTGACCGC AACCTTCCCG TGGGGCTAAG 240

GCAAAAGAGC CTGACAGAGA AAACCCCCAC AGGGACATTC AGCAGAGAGG CACTGATGGC 300

CTATTGGGAA AAGGAGTCCC AAAAACTCTT GGAGAAGGAG AGGCTGGGGG AATGTGGAAA 360

GGTTGCAGAA GACAAAGAGG AAAGTGAAGA AGAGCTTATC TTTACTGAAA GTAACAGTGA 420

GGTTTCTGAG GAAGTGTATA CAGAGGAGGA GGAGGAGGAG TCCCAGGAGG AAGAGGAGGA 480

AGAAGACAGT GACGAAGAGG AAAGAACAAT TGAAACTGCA AAAGGGATTA ATGGAACTGT 540

AAATTATGAT AGTGTCAATT CTGACAACTC TAAGCCAAAG ATATTTAAAA GTCAAATAGA 600

GAACATAAAT TTGACCAATG GCAGCAATGG GAGGAACACA GAGTCCCCAG CTGCCATTCA 660

CCCTTGTGGA AATCCTACAG TGATTGAGGA CGCTTTGGAC AAGATTAAAA GCAATGACCC 720

TGACACCACA GAAGTCAATT TGAACAACAT TGAGAACATC ACAACACAGA CCCTTACCCG 780

CTTTGCTGAA GCCCTCAAGG ACAACACTGT GGTGAAGACG TTCAGTCTGG CCAACACGCA 840

TGCCGACGAC AGTGCAGCCA TGGCCATTGC AGAGATGCTC AAAGCCAATG AGCACATCAC 900

CAACGTAAAC GTCGAGTCCA ACTTCATAAC GGGAAAGGGG ATCCTGGCCA TCATGAGAGC 960

TCTCCAGCAC AACACGGTGC TCACGGAGCT GCGTTTCCAT AACCAGAGGC ACATCATGGG 1020

CAGCCAGGTG GAAATGGAGA TTGTCAAGCT GCTGAAGGAG AACACGACGC TGCTGAGGCT 1080

GGGATACCAT TTTGAACTCC CAGGACCAAG AATGAGCATG ACGAGCATTT TGACAAGAAA 1140

TATGGATAAA CAGAGGCAAA AACGTTTGCA GGAGCAAAAA CAGCAGGAGG GATACGATGG 1200

AGGACCCAAT CTTAGGACCA AAGTCTGGCA AAGAGGAACA CCTAGCTCTT CACCTTATGT 1260

ATCTCCCAGG CACTCACCCT GGTCATCCCC AAAACTCCCC AAAAAAGTCC AGACTGTGAG 1320

GAGCCGTCCT CTGTCTCCTG TGGCCACACT TCCTCCTCCT CCCCCTCCTC CTCCTCCTCC 1380

CCCTCCTTCT TCCCAAAGGC TGCCACCACC TCCTCCTCCT CCCCCTCCTC CACTCCCAGA 1440

GAAAAAGCTC ATTACCAGAA ACATTGCAGA AGTCATCAAA CAACAGGAGA GTGCCCAACG 1500

GGCATTACAA AATGGACAAA AAAAGAAAAA AGGGAAAAAG GTCAAGAAAC AGCCAAACAG 1560

TATTCTAAAG GAAATAAAAA ATTCTCTGAG GTCAGTGCAA GAGAAGAAAA TGGAAGACAG 1620

TTCCCGACCT TCTACCCCAC AGAGATCAGC TCATGAGAAT CTCATGGAAG CAATTCGGGG 1680

AAGCAGCATA AAACAGCTAA AGCGGGTGGA AGTTCCAGAA GCCCTGCGAT GGGAACATGA 1740

TCTTTAGAAG AGGATGCAGA ACTGTTCAGT GGTATTACAT GAAATGCATT GTGAGATGTT 1800

TCTAAAATAC CTTCTTCAAT TCAAAATGAT CCCTGACTTT AAAAATAATC TCACCCATTA 1860

ATTCCAAAGA GAATCTTAAG AAACAATCAG CATGTTTCTT CTGTAAATAT GAAAATAAAT 1920

TTCTTTTTTA TGTCGT 1936

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2080 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: YES

(vi) ORIGINAL SOURCE:
(F) TISSUE TYPE: cardiac tissue

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 2:

```
CAGCCTGCCA CTTGCCTCCC TGCCTGCTTC TGGCTGCCTT GAATGCCTGG TCCTTCAAGC      50
TCCTTCTGGG TCTGACAAAG CAGGGACCAT GTCTACCTTT GGCTACCGAA GAGGACTCAG     120
TAAATACGAA TCCATCGACG AGGATGAACT CCTCGCCTCC CTGTCAGCCG AGGAGCTGAA     180
GGAGCTAGAG AGAGAGTTGG AAGACATTGA ACCTGACCGC AACCTTCCCG TGGGGCTAAG     240
GCAAAAGAGC CTGACAGAGA AAACCCCCAC AGGGACATTC AGCAGAGAGG CACTGATGGC     300
CTATTGGGAA AAGGAGTCCC AAAAACTCTT GGAGAAGGAG AGGCTGGGGG AATGTGGAAA     360
GGTTGCAGAA GACAAAGAGG AAAGTGAAGA AGAGCTTATC TTTACTGAAA GTAACAGTGA     420
GGTTTCTGAG GAAGTGTATA CAGAGGAGGA GGAGGAGGAG TCCCAGGAGG AAGAGGAGGA     480
AGAAGACAGT GACGAAGAGG AAAGAACAAT TGAAACTGCA AAAGGGATTA ATGGAACTGT     540
AAATTATGAT AGTGTCAATT CTGACAACTC TAAGCCAAAG ATATTTAAAA GTCAAATAGA     600
GAACATAAAT TTGACCAATG GCAGCAATGG GAGGAACACA GAGTCCCCAG CTGCCATTCA     660
CCCTTGTGGA AATCCTACAG TGATTGAGGA CGCTTTGGAC AAGATTAAAA GCAATGACCC     720
TGACACCACA GAAGTCAATT TGAACAACAT TGAGAACATC ACAACACAGA CCCTTACCCG     780
CTTTGCTGAA GCCCTCAAGG ACAACACTGT GGTGAAGACG TTCAGTCTGG CCAACACGCA     840
TGCCGACGAC AGTGCAGCCA TGGCCATTGC AGAGATGCTC AAAGCCAATG AGCACATCAC     900
CAACGTGAAC GTCGAGTCCA ACTTCATAAC GGGAAAGGGG ATCCTGGCCA TCATGAGAGC     960
TCTCCAGCAC AACACGGTGC TCACGGAGCT GCGTTTTCAT AACCAGAGGC ACATCATGGG    1020
CAGCCAGGTG GAAATGGAGA TTGTCAAGCT GCTGAAGGAG AACACGACGC TGCTGAGGCT    1080
GGGATACCAT TTTGAACTCC CAGGACCAAG AATGAGCATG ACGAGCATTT TGACAAGAAA    1140
TATGGATAAA CAGAGGCAAA AACGTTTGCA GGAGCAAAAA CAGCAGGAGG GATACGATGG    1200
AGGACCCAAT CTTAGGACCA AAGTCTGGCA AAGAGGAACA CCTAGCTCTT CACCTTATGT    1260
ATCTCCCAGG CACTCACCCT GGTCATCCCC AAAACTCCCC AAAAAAGTCC AGACTGTGAG    1320
GAGCCGTCCT CTGTCTCCTG TGGCCACACT TCCTCCTCCT CCCCCTCCTC CTCCTCCTCC    1380
CCCTCCTTCT TCCCAAAGGC TGCCACCACC TCCTCCTCCT CCCCCTCCTC CACTCCCAGA    1440
GAAAAAGCTC ATTACCAGAA ACATTGCAGA AGTCATCAAA CAACAGGAGA GTGCCCAACG    1500
GGCATTACAA AATGGACAAA AAAAGAAAAA AGGGAAAAAG GTCAAGAAAC AGCCAAACAG    1560
TATTCTAAAG GAAATAAAAA ATTCTCTGAG GTCAGTGCAA GAGAAGAAAA TGGAAGACAG    1620
TTCCCGACCT TCTACCCCAC AGAGATCAGC TCATGAGAAT CTCATGGAAG CAATTCGGGG    1680
AAGCAGCATA AAACAGCTAA AGCGGGTGGA AGTTCCAGAA GCCCTGCGAT GGGAACATGA    1740
TCTTTAGAAG AGGATGCAGA ACTGTTCAGT GGTATTACAT GAAATGCATT GTGAGATGTT    1800
TCTAAAATAC CTTCTTCAAT TCAAAATGAT CCCTGACTTT AAAAATAATC TCACCCATTA    1860
ATTCCAAAGA GAATCTTAAG AAACAATCAG CATGTTTCTT CTGTAAATAT GAAAATAAAT    1920
TTCTTTTTTA TGTCGTGAGA TTTGTATTGG CAAGAAGCAG TTAATTTAAA GATGCTCTTC    1980
CTATCTGTGG ATGTGTTGGT AACTCCGAGT TGTAATGAGT TCATGAAATG TGCTGTTATT    2040
TTTGTAATCT CAATAAATGT GGATTGAAGT TTTTTCCCTT                          2080
```

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2268 base pairs

(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: YES

(vi) ORIGINAL SOURCE:
(F) TISSUE TYPE: cardiac tissue

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 3:

CAGCCTGCCA CTTGCCTCCC TGCCTGCTTC TGGCTGCCTT GAATGCCTGG TCCTTCAAGC 60
TCCTTCTGGG TCTGACAAAG CAGGGACCAT GTCTACCTTT GGCTACCGAA GAGGACTCAG 120
TAAATACGAA TCCATCGACG AGGATGAACT CCTCGCCTCC CTGTCAGCCG AGGAGCTGAA 180
GGAGCTAGAG AGAGAGTTGG AAGACATTGA ACCTGACCGC AACCTTCCCG TGGGGCTAAG 240
GCAAAAGAGC CTGACAGAGA AAACCCCCAC AGGGACATTC AGCAGAGAGG CACTGATGGC 300
CTATTGGGAA AAGGAGTCCC AAAAACTCTT GGAGAAGGAG AGGCTGGGGG AATGTGGAAA 360
GGTTGCAGAA GACAAAGAGG AAAGTGAAGA AGAGCTTATC TTTACTGAAA GTAACAGTGA 420
GGTTTCTGAG GAAGTGTATA CAGAGGAGGA GGAGGAGGAG TCCCAGGAGG AAGAGGACGA 480
AGAAGACAGT GACGAAGAGG AAAGAACAAT TGAAACTGCA AAAGGGATTA ATGGAACTGT 540
AAATTATGAT AGTGTCAATT CTGACAACTC TAAGCCAAAG ATATTTAAAA GTCAAATAGA 600
GAACATAAAT TTGACCAATG GCAGCAATGG GAGGAACACA GAGTCCCCAG CTGCCATTCA 660
CCCTTGTGGA AATCCTACAG TGATTGAGGA CGCTTTGGAC AAGATTAAAA GCAATGACCC 720
TGACACCACA GAAGTCAATT TGAACAACAT TGAGAACATC ACAACACAGA CCCTTACCCG 780
CTTTGCTGAA GCCCTCAAGG ACAACACTGT GGTGAAGACG TTCAGTCTGG CCAACACGCA 840
TGCCGACGAC AGTGCAGCCA TGGCCATTGC AGAGATGCTC AAAGCCAATG AGCACATCAC 900
CAACGTAAAC GTCGAGTCCA ACTTCATAAC GGGAAAGGGG ATCCTGGCCA TCATGAGAGC 960
TCTCCAGCAC AACACGGTGC TCACGGAGCT GCGTTTCCAT AACCAGAGGC ACATCATGGG 1020
CAGCCAGGTG GAAATGGAGA TTGTCAAGCT GCTGAAGGAG AACACGACGC TGCTGAGGCT 1080
GGGATACCAT TTTGAACTCC CAGGACCAAG AATGAGCATG ACGAGCATTT TGACAAGAAA 1140
TATGGATAAA CAGAGGCAAA AACGTTTGCA GGAGCAAAAA CAGCAGGAGG GATACGATGG 1200
AGGACCCAAT CTTAGGACCA AAGTCTGGCA AAGAGGAACA CCTAGCTCTT CACCTTATGT 1260
ATCTCCCAGG CACTCACCCT GGTCATCCCC AAAACTCCCC AAAAAAGTCC AGACTGTGAG 1320
GAGCCGTCCT CTGTCTCCTG TGGCCACACT TCCTCCTCCT CCCCCTCCTC CTCCTCCTCC 1380

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CCCTCCTTCT TCCCAAAGGC TGCCACCACC TCCTCCTCCT CCCCCTCCTC CACTCCCAGA  144C
GAAAAAGCTC ATTACCAGAA ACATTGCAGA AGTCATCAAA CAACAGGAGA GTGCCCAACG  150C
GGCATTACAA AATGGACAAA AAAAGAAAAA AGGGAAAAAG GTCAAGAAAC AGCCAAACAG  156C
TATTCTAAAG GAAATAAAAA ATTCTCTGAG GTCAGTGCAA GAGAAGAAAA TGGAAGACAG  162C
TTCCCGACCT TCTACCCCAC AGAGATCAGC TCATGAGAAT CTCATGGAAG CAATTCGGGG  168C
AAGCAGCATA AAACAGCTAA AGCGGGTGGA AGTTCCAGAA GCCCTGCGAT GGGAACATGA  174C
TCTTTAGAAG AGGATGCAGA ACTGTTCAGT GGTATTACAT GAAATGCATT GTGAGATGTT  180C
TCTAAAATAC CTTCTTCAAT TCAAAATGAT CCCTGACTTT AAAAATAATC TCACCCATTA  186C
ATTCCAAAGA GAATCTTAAG AAACAATCAG CATGTTTCTT CTGTAAATAT GAAAATAAAT  192C
TTCTTTTTTA TGTCGTGAGA TTTGTATTGG CAAGAAGCAG TTAATTTAAA GATGCTCTTC  198C
CTATCTGTGG ATGTGTTGGT AACTCCGAGT TGTAATGAGT TCATGAAATG TGCTGTTATT  204C
TTTGTAATCT CAATAAATGT GGATTGAAGT TTTTTCCCTT TTTTTAAAGC CAAACTAATA  210C
TTTTTCTGTG ACTTGATACA TCTGTCAGAT TTTTGTAATC TCGATAAATG TGTATTGAAG  216C
TTTTTTCCCT TTTTTTAAAA AGCCAAACTA ATATTTTTCT GTGAGTTAAT ACATCTGTCA  222C
GGTGTGTATG TAACATTACT GGACATTAAA AAAAATTATT ACATTCTC               225C
```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 552 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: YES

(vi) ORIGINAL SOURCE:
(F) TISSUE TYPE: cardiac tissue

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 4:

```
Met Ser Thr Phe Gly Tyr Arg Arg Gly Leu Ser Lys Tyr Glu Ser Ile
1               5                   10                  15

Asp Glu Asp Glu Leu Leu Ala Ser Leu Ser Ala Glu Glu Leu Lys Glu
            20                  25                  30

Leu Glu Arg Glu Leu Glu Asp Ile Glu Pro Asp Arg Asn Leu Pro Val
        35                  40                  45

Gly Leu Arg Gln Lys Ser Leu Thr Glu Lys Thr Pro Thr Gly Thr Phe
    50                  55                  60
```

Ser Arg Glu Ala Leu Met Ala Tyr Trp Glu Lys Glu Ser Gln Lys Leu
65 70 75 80

Leu Glu Lys Glu Arg Leu Gly Glu Cys Gly Lys Val Ala Glu Asp Lys
85 90 95

Glu Glu Ser Glu Glu Glu Leu Ile Phe Thr Glu Ser Asn Ser Glu Val
100 105 110

Ser Glu Glu Val Tyr Thr Glu Glu Glu Glu Glu Glu Ser Gln Glu Glu
115 120 125

Glu Glu Glu Glu Asp Ser Asp Glu Glu Glu Arg Thr Ile Glu Thr Ala
130 135 140

Lys Gly Ile Asn Gly Thr Val Asn Tyr Asp Ser Val Asn Ser Asp Asn
145 150 155 160

Ser Lys Pro Lys Ile Phe Lys Ser Gln Ile Glu Asn Ile Asn Leu Thr
165 170 175

Asn Gly Ser Asn Gly Arg Asn Thr Glu Ser Pro Ala Ala Ile His Pro
180 185 190

Cys Gly Asn Pro Thr Val Ile Glu Asp Ala Leu Asp Lys Ile Lys Ser
195 200 205

Asn Asp Pro Asp Thr Thr Glu Val Asn Leu Asn Asn Ile Glu Asn Ile
210 215 220

Thr Thr Gln Thr Leu Thr Arg Phe Ala Glu Ala Leu Lys Asp Asn Thr
225 230 235 240

Val Val Lys Thr Phe Ser Leu Ala Asn Thr His Ala Asp Asp Ser Ala
245 250 255

Ala Met Ala Ile Ala Glu Met Leu Lys Ala Asn Glu His Ile Thr Asn
260 265 270

Val Asn Val Glu Ser Asn Phe Ile Thr Gly Lys Gly Ile Leu Ala Ile
275 280 285

Met Arg Ala Leu Gln His Asn Thr Val Leu Thr Glu Leu Arg Phe His
290 295 300

Asn Gln Arg His Ile Met Gly Ser Gln Val Glu Met Glu Ile Val Lys
305 310 315 320

Leu Leu Lys Glu Asn Thr Thr Leu Leu Arg Leu Gly Tyr His Phe Glu
325 330 335

Leu Pro Gly Pro Arg Met Ser Met Thr Ser Ile Leu Thr Arg Asn Met
340 345 350

Asp Lys Gln Arg Gln Lys Arg Leu Gln Glu Gln Lys Gln Gln Glu Gly
355 360 365

Tyr Asp Gly Gly Pro Asn Leu Arg Thr Lys Val Trp Gln Arg Gly Thr
370 375 380

Pro Ser Ser Ser Pro Tyr Val Ser Pro Arg His Ser Pro Trp Ser Ser
385 390 395 400

Pro Lys Leu Pro Lys Lys Val Gln Thr Val Arg Ser Arg Pro Leu Ser
405 410 415

Pro Val Ala Thr Leu Pro Pro Pro Pro Pro Pro Pro Pro Pro Pro Pro
420 425 430

Pro Ser Ser Gln Arg Leu Pro Pro Pro Pro Pro Pro Pro Pro Pro Pro
435 440 445

Leu Pro Glu Lys Lys Leu Ile Thr Arg Asn Ile Ala Glu Val Ile Lys
450 455 460

Gln Gln Glu Ser Ala Gln Arg Ala Leu Gln Asn Gly Gln Lys Lys Lys
465 470 475 480

Lys Gly Lys Lys Val Lys Lys Gln Pro Asn Ser Ile Leu Lys Glu Ile
485 490 495

Lys Asn Ser Leu Arg Ser Val Gln Glu Lys Lys Met Glu Asp Ser Ser
500 505 510

Arg Pro Ser Thr Pro Gln Arg Ser Ala His Glu Asn Leu Met Glu Ala
515 520 525

Ile Arg Gly Ser Ser Ile Lys Gln Leu Lys Arg Val Glu Val Pro Glu
530 535 540

Ala Leu Arg Trp Glu His Asp Leu
545 550

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: YES

(vi) ORIGINAL SOURCE:
(F) TISSUE TYPE: cardiac tissue

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 5

CCTTCTACCC 1:

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 279 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: YES

(vi) ORIGINAL SOURCE:
(F) TISSUE TYPE: cardiac tissue

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 6:

```
GCCAACACGC  ANTCCGACGA  CAGTGCAGCC  ATGGTCATTG  CAGAGATGCN  TCAAAGTCAA  60
TGAGCACATC  ACCAACGTAA  ACGTCGAGTC  CAACTTCATA  ACGGGAAAGG  GGATCCTGGC  120
CATCATGAGA  GCTCTCCAGC  ACAACACGGT  GCTCACGGAG  CTGCGGTTTC  ATAACCAGAG  180
GCACATCATG  GGCAGCCAGG  TGGAAATGGA  GATTGTCAAG  CTNCTGAAGG  AGAACACGAC  240
GCTNCTGAGG  CTGGGNTACC  ATTTTNAACT  CCCAGGACC                          279
```

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 93 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: YES

(vi) ORIGINAL SOURCE:
(F) TISSUE TYPE: cardiac tissue

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 7:

```
PTRNPTTVQPWSLQRCIKVNEHITNVNVESNFITGKGILAIMRALQ
          10        20        30        40
HNTVLTELRFHNQRHIMGSQVEMEIVKLLKENTTLLRLGYHFKLPG
  50        60        70        80        90
```